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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/542,117	11/30/2005	Lily Yang	14507-50900	4006

24728 7590 08/15/2008

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EXAMINER	
BRISTOL, LYNN ANNE	
ART UNIT	PAPER NUMBER
1643	
MAIL DATE	DELIVERY MODE
08/15/2008	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/542,117	YANG ET AL.	
	Examiner	Art Unit	
	LYNN BRISTOL	1643	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 May 2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-17 and 50-54* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-17 and 50-54* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *2/21/08*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Claims 1-17 and 50-54 are all the pending claims for this application.

2. Claims 18-49 were cancelled, Claims 1-3 and 9-17 were amended and new Claims 50-54 were added in the Response 5/2/08.

3. The non-elected species of tumor marker in Claim 2 are withdrawn from examination.

4. Claims 1-17 and 50-54 are all the pending claims under examination with species of tumor marker for survivin (and oligonucleotide probes of SEQ ID NOS:1, 2 and 9), cyclin D1 (and oligonucleotide probes of SEQ ID NOS: 3 and 4), Her2/neu (and oligonucleotide probes of SEQ ID NOS: 5 and 6), a mutant K-ras (and oligonucleotide probes of SEQ ID NOS: 7, 8, 11, 12 and 13), chymotrypsinogen, carcinoembryonic antigen and human chorionic gonadotropin.

5. Applicants amendments to the claims have necessitated new grounds for objection and rejection. This action is FINAL.

Information Disclosure Statement

6. The IDS from 2/21/08 has been considered and entered. The copy of the examiner's initialed 1449 form is attached.

Withdrawal of Objections

Claim Objections

7. The objections to Claims 2 and 3 for informalities are withdrawn for the following reasons:

a) the numerous typographical errors in the spelling of the turmor marker species in Claim 2, for example, "basic fiborblast growth factor"; "prostate, specific antigen"; and "alpha-fetalprotein" have been corrected by amendment.

b) The recitation "the sample taken from" in Claim 3 has been amended to "the sample is taken from...".

Withdrawal of Rejections

Claim Rejections - 35 USC § 112, second paragraph

8. The rejection of Claims 1-17 in lacking antecedent basis for the limitation "the target sequence" in elements iii) and iv) of Claim 1 is withdrawn in view of the deletion of the limitation from Claim 1.

9. The rejection of Claims 1-17 for the recitation "a probing nucleobase sequence" in Claim 1 is withdrawn in view of the amendment of Claim 1 to describe the nucleobase sequence as being complementary to a target sequence in the tumor marker mRNA.

10. The rejection of Claim 9 for the recitation "the oligonucleotide is SEQ ID NO:10" which corresponds to the housekeeping gene, GAPDH, which is not a tumor marker, is withdrawn. Claim 9 has been amended to delete the sequence identifier from the claim.

11. The rejection of Claim 13 for reciting improper Markush group language is withdrawn.

12. The rejection of Claims 1-17 for the limitation "the hybridization of the target sequence under suitable hybridization conditions" in Claim 1 is withdrawn in view of the amendment of Claim 1 to delete the phrase. However, the amended claim raises new grounds for rejection set forth below.

Claim Rejections - 35 USC § 102

13. The rejection of Claims 1, 2, 3, 4 and 5 under 35 U.S.C. 102(a) as being anticipated by Span et al. (Clin. Chem. 49(7); 1074-1080 (July 2003)) is withdrawn.

Applicants' allegations on p. 9 of the Response of 5/2/08 have been considered and are found persuasive. Applicants allege that Span does not teach tumor marker mRNA detection by incubating a cell with the molecular beacon probes but instead extracts the mRNA from the cell or tissue prior to the hybridization/detection step. Amended Claim 1 requires using a cell from a sample in which to detect tumor marker mRNA.

14. The rejection of Claims 1, 2, 3 and 14 under 35 U.S.C. 102(a) as being anticipated by Vijayanathan et al. (Antisense & Nuc. Acid drug Devel. 12:225-233 August 2002)) is withdrawn.

Applicants' allegations on p. 9 of the Response of 5/2/08 have been considered and are found persuasive. Applicants allege that Vijayanathan does not teach tumor marker mRNA detection by incubating a cell with the molecular beacon probes but instead uses a synthetic mRNA in the hybridization/detection step. Amended Claim 1 requires using a cell from a sample in which to detect tumor marker mRNA.

15. The rejection of Claims 1, 2, 3, 4 and 16 under 35 U.S.C. 102(a) as being anticipated by Singer et al. (Am. J. Pathol. 160(4):1223-1228 (April 2002)) as evidenced by Vogelstein et al. (PNAS 96:9236-9241 (1999)) is withdrawn.

Applicants' allegations on p. 9 of the Response of 5/2/08 have been considered and are found persuasive. Applicants allege that Singer and Vogelstein require extraction of DNA or mRNA from a cell and detection of tumor markers by RT-PCR. Applicants allege Singer and Vogelstein do not teach incubating a molecular beacon with a cell as recited in Claim 1.

16. The rejection of Claims 1, 2, 3, and 10 under 35 U.S.C. 102(b) as being anticipated by Wen et al. (Cancer Gene Ther. 7(11):1469-1480 (2000)) is withdrawn.

Applicants' allegations on p. 9 of the Response of 5/2/08 have been considered and are found persuasive. Applicants allege that Wen requires extraction of mRNA from

a tumor cell sample and detection of tumor markers by RT-PCR instead of directly contacting the cell with the beacon probes.

17. The rejection of Claims 1, 2, 3, 4, 7, 8, 9, 10, 16 and 17 under 35 U.S.C. 102(e) as being anticipated by Bao et al. (US 20060127940; published 6/15/06; filed 6/25/02) is withdrawn.

Applicants' allegations on p. 10 of the Response of 5/2/08 have been considered and are found persuasive. Applicants allege that Bao teaches a dual molecular beacon method that combines two molecular beacons each with a single resonance energy transfer method.

18. The rejection of Claims 1, 2, 3, 4, 6, 12, and 14 under 35 U.S.C. 102(e) as being anticipated by Harbeck et al. (US 20060084056; published 4/20/2006; priority to 2/13/2002) is withdrawn.

Applicants' allegations on p. 9 of the Response of 5/2/08 have been considered. Applicants allege that Harbeck requires extraction of mRNA from a tumor cell sample and detection of tumor markers by RT-PCR instead of directly contacting the cell with the beacon probes.

The examiner submits that Harbeck teaches "Additionally, it is possible to measure the levels of uPA and PAI-1 using in situ assays, i.e., directly upon tissue sections (fixed and/or frozen, e.g., paraffin sections) of patient tissue obtained from biopsies or resections, (e.g., laser micro-dissection of single cells) such that no nucleic

acid purification is necessary. Noack et al., 1999, Int. J. Oncol. 15(4):617-23. Nucleic acid reagents such as those described in Section 5.7 may be used as probes and/or primers for such in situ procedures (see, e.g., Nuovo, G. J., 1992, PCR In Situ Hybridization: Protocols And Applications, Raven Press, NY)" [0179]. Thus contrary to Applicants allegations, Harbeck teaches and appreciates performing detection methods using the molecular beacon probes by directly contacting cells in situ which does not require mRNA extraction. Further, it is the examiner's position that the instant claims do not exclude a PCR step because they recite "comprising" language and that the only requirement of the probe is that it hybridizes to the corresponding mRNA.

However, Harbeck does not disclose the specific beacon probes having the structure(s) as instantly claimed in Claim 1, and therefore the rejection is withdrawn.

19. The rejection of Claims 1, 2, 3, 4, 7, 8, 9, 10, 16 and 17 under 35 U.S.C. 102(e) as being anticipated by Bao et al. (USPN 7297494; published 11/20/07; priority to 6/25/2001 for K-ras mutant molecular beacon) is withdrawn.

Applicants' allegations on p. 10 of the Response of 5/2/08 have been considered and are found persuasive. Applicants allege that Bao teaches a dual molecular beacon method that combines two molecular beacons each with a single resonance energy transfer method.

Objections Maintained

Claim Objections

20. The objection to Claim 16 is maintained. The original recitation "targets-the tumor-marker a K-ras mutant gene" in Claim 16 has now been amended to "the tumor markers comprise a K-ras mutant gene", and the amendment does not address the original issue. Claim 2 recites "a mutant K-ras" and it is unclear if this is a typographical error.

Rejections Maintained

Claim Rejections - 35 USC § 112, second paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

21. The rejection of Claim 16 for the recitation "the one or more tumor markers comprise a K-ras mutant gene" is maintained. The method of claim 1 is drawn to detecting tumor marker mRNA with the oligonucleotide, whereas Claim 16 is drawn to targeting the gene for a mutant K-ras. The specification at p. 14, lines 12-16 describes the method as targeting the K-ras mutant gene with the oligonucleotides and at line 24 as targeting the mRNA for a mutant K-ras. Is a different method step required in Claim 16 that requires gene targeting versus targeting mRNA?

Alternatively, the method step in Claim 16 is a broadening limitation for detecting a gene whereas the generic method of Claim 1 is drawn to detecting "tumor marker mRNA".

New Grounds for Objection

Claim Objections

22. Claims 1 and 50 are objected to for an apparent spelling error:

a) In step (ii) of Claim 1, "one or more nucleotide" should seemingly recite "one or more nucleotides";

b) Claim 50 recites "surviving" and should seemingly recite "survivin."

New Grounds for Rejection

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

23. Claims 1-17 and 50-54 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

a) Claims 1-17 are indefinite for the recitation "one or more oligonucleotides that hybridize to the mRNA of one or more tumor markers" and "wherein each oligonucleotide hybridizes to the mRNA of a different tumor marker and emits a fluorescent signal after hybridization with the corresponding mRNA" in Claim 1 because the meaning of the term "hybridize" or "hybridizes" or "hybridization" is not clear from the meaning of the claims or the specification. The claims and the specification do not describe any general set of hybridization conditions much less specific hybridization conditions for the genus of tumor-marker specific oligonucleotides to hybridize in situ

within a cell and one of skill in the art would not be able to determine what conditions Applicant intended the claim to encompass. Since the specification does not provide an unambiguous definition or description for the simultaneous hybridization of the "one or more oligonucleotides" in a cell encompassed by the claims, the metes and bounds of the claimed invention are not known.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Enablement

24. Claims 1-17 and 50-54 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for detecting tumor mRNA of one or more tumor markers in a test sample cell compared to a control cell with molecular beacon probes having complementarity to the corresponding mRNA and further comprising different fluorophors in order to distinguish the mRNA species, does not reasonably provide enablement for a) practicing the method in the absence of a control cell in order to substract background autofluorescence (and determine signal/ noise ratio) or b) using different oligonucleotides complementary to a corresponding mRNA but comprising the same energy donor moiety and the same energy acceptor moiety (i.e., probes complementary to different tumor mRNA species but having the same 5' fluorophor and the same 3' fluorophor). The specification does not enable any

person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required are summarized in In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988). They include the nature of the invention, the state of the prior art, the relative skill of those in the art, the amount of direction or guidance disclosed in the specification, the presence or absence of working examples, the predictability of the art, the breadth of the claims, the quantity of experimentation which would be required in order to use the invention as claimed.

Nature of the Invention/ Skill in the Art

The claims are *interpreted* as being drawn to a method for detecting the presence of tumor marker mRNA in cells, tissues or bodily fluids using one or more oligonucleotides that hybridize to one or more tumor markers and the oligos are molecular beacon probes complementary to a sequence in the mRNA and each comprises a 5' moiety and a 3' moiety that are not necessarily different between the different probes. The claims are drawn to the elected species of tumor markers for survivin (and oligonucleotide probes of SEQ ID NOS:1, 2 and 9), cyclin D1 (and oligonucleotide probes of SEQ ID NOS: 3 and 4), Her2/neu (and oligonucleotide probes of SEQ ID NOS: 5 and 6), a mutant K-ras (and oligonucleotide probes of SEQ ID NOS: 7, 8, 11, 12 and 13), chymotrypsinogen, carcinoembryonic antigen and human chorionic gonadotropin.

This means that the claims are drawn to an assay of (1) any tissue/cell/bodily

fluid where a control for autofluorescence is missing, wherein any measured level of a fluorescence signal is associated with the presence of a tumor marker mRNA, (2) this also means that the test sample is not required to comprise tumor cells, and also means (3) the detection of any fluorescent signal comprising assaying any cell using the instant method wherein an increase in measured levels of a fluorescent signal is associated with the presence of a tumor marker mRNA would not allow one to distinguish one tumor marker mRNA from a different tumor marker mRNA because the energy donor moiety and energy acceptor moiety are not required to be different for the "one or more oligonucleotides".

The relative skill in the art required to practice the invention is a pathology technician with a background in situ hybridization and fluorescent whole cell visualization.

Disclosure in the Specification/ Art Status

One cannot extrapolate the teaching of the specification to the scope of the claims because (1) given the teaching of the specification one would not be able to predictably and specifically detect a tumor marker mRNA by merely measuring any fluorescent signal in any kind of cell in the absence of a control cell to determine the level for autofluorescence background, (2) in the absence of assaying tumor cells one would not expect to determine the presence of any tumor marker mRNA, and (3) given the teaching of the specification, it is clear that different fluorophors on the species of oligonucleotides are required to distinguish one species of probe/mRNA hybrid from another hybrid species and one could not predictably detect a differential of expression

of different tumor marker mRNAs where the probes shared the same 5' and 3' fluorophors.

(1) The broadly claimed invention encompasses the fluorescent signal detection of tumor marker mRNA assaying any cell wherein the specification makes absolutely clear that matched cancer and control cells are required for correlating the level of fluorescence intensity (assessing signal to noise ratio). In Example 1, survivin molecular beacon was examined in 3 breast cancer cell lines and normal human mammary epithelial cell line and results shown in Figure 4 reveal that the fluorescence is intermediate to strong in the cancer cell lines, but that still a weak fluorescence signal is detected in the normal control. Unless one were to specifically make a comparison to a control cell/tissue, it would be expected that fluorescence signal with the test samples alone could produce false positive determinations for the presence of tumor marker mRNA (and detection of cancer). A control cell would be necessary when the cell emits detectable autofluorescence or to determine the signal/noise ratio for fluorescence from non-specific hybridization of the probe with cellular components. Sokol et al. (PNAS 95:11538-11543 (1998); cited in the IDS of 2/21/08) addresses the stability of molecular beacon (MB) probes in an intracellular environment to find that a control MB could emit fluorescent signals upon exposure to 45 minutes of UV light and that degradation of MB/target duplexes might explain this non-specific fluorescence.

(2) The claims as currently constituted do not require that cells that are assayed are tumor cells and in the absence of assaying tumor cells, it is not clear how one could detect any tumor marker mRNA with the instantly broadly claimed method because the

claims read on assaying any cells, tissues or bodily fluids, regardless of their pathological state. Thus one could not predict that the claimed invention will function as claimed with a reasonable expectation of success. In particular the specification teaches, as drawn to an assay for the detection of cancerous cells in a sample, that cancer patient tissues were already pre-determined to express the proteins for the tumor marker mRNAs (See Example 3 and 4). Clearly, critical to the detection of tumor marker mRNA is the assay of tumor cells. Sokol et al. (PNAS 95:11538-11543 (1998); cited in the IDS of 2/21/08) teaches specificity using control MB probes to assess background noise and treatment of cells that do not express the message and "In all cases, signal clearly above background was observed under those condition in which it was expected, i.e., when the MB and its targeted complement were present in the cell under study" (p. 11541, Col. 2, ¶3).

(3) The claims encompass molecular beacon oligonucleotide probes that are required to be different in their sequence structure in order to distinguish one species of tumor marker mRNA from another species (i.e., to be complementary), but which otherwise would produce the same fluorescence signal as a readout. This is because none of the claims describe or require that the energy donor and energy acceptor moiety should differ between any "one or more of the oligonucleotides". Here the specification teaches by way of example in Table 1, that to simultaneously visualize hybridizations between distinct species of complementary probe/mRNA within the same cell, the probe is labeled with different fluorophors. For example, Yang et al. (Can. Biol. Therap. 4(5): 561-570 (2005); cited in the IDS of 2/21/08) demonstrated that molecular

beacons labeled with different fluorophors could detect surivin and mutant K-ras mRNAs simultaneously in single cancer cells and Bratu et al. (PNAS 100 (23):13308-13313 (2003); cited in the IDS of 2/21/08) teaches different mRNAs within a cell can be distinguished from one another by using molecular beacons that are specific for different mRNAs labeled with differently colored fluorophors.

The specification provides no working examples which would provide guidance to one skilled in the art and no evidence has been provided which would allow one of skill in the art to predict that the invention as currently claimed would function as claimed, with a reasonable expectation of success. For the above reasons, it appears that undue experimentation would be required to practice the claimed invention."

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Rav oncogene/ urokinase-type plasminogen activator (uPA)/ plasminogen activator inhibitor-1 (PAI-1)/ cyclin D1/ carcinoembryonic antigen/ Her2/neu

25. Claims 1-6, 12 and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sokol et al. (PNAS 95:11538-11543 (1998); cited in the IDS of 2/21/08) in view of Harbeck et al. (US 20060084056; published 4/20/2006; priority to 2/13/2002; cited in the PTO 892 form of 2/5/08).

Claim 1 is interpreted as being drawn to a method for detecting the presence of tumor marker mRNA in cells using one or more oligonucleotides that hybridize to one or more tumor markers and the oligos are molecular beacon probes complementary to a sequence in the mRNA and each comprises a 5' energy donor moiety and a 3' energy acceptor moiety. The claims are drawn to the elected species of tumor markers for cyclin D1, Her2/neu and carcinoembryonic antigen of Claim 2, Claim 3 is drawn to the sample from breast lavage, aspiration or needle biopsy, Claim 4 is drawn to a breast biopsy, Claim 5 is drawn to frozen tissue, Claim 6 is drawn to breast lavage, Claim 12 is drawn to the oligo targeting cyclin D1 and Claim 14 is drawn to the oligo targeting Her2/neu.

The claimed method was prima facie obvious at the time of the invention over Sokol in view of Harbeck.

Sokol teaches real-time detection of molecular beacon (MB) target mRNA hybridization in living cells by microinjecting a MB to *rav* protooncogene or a control MB into K562 human leukemia cells, and that by fluorescence microscopic detection within 15 min, cells were fluorescent for the rav MB but not the control. Sokol's MB consisted

of a 24 nucleotide loop sequence flanked on the 5' and 3' ends with a fluorescent donor (EDANS) and acceptor (DABCYL), respectively (Figure 1). Sokol teaches that once the MB hybridizes with its respective endogenous complementary mRNA target the fluorophor emits light which can be readily detected by fluorimetry means (p. 11540, Col. 2, ¶3- p.11541, Col. 1; Figure 3). Sokol teaches specificity using control MB probes to assess background noise and treatment of cells that do not express the message and "In all cases, signal clearly above background was observed under those condition in which it was expected, i.e., when the MB and its targeted complement were present in the cell under study" (p. 11541, Col. 2, ¶3). Sokol teaches and appreciates that MBs can be used to study many aspects of RNA biology in different kinds of cells, but does not specifically teach detecting markers for urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor-1 (PAI-1) in combination with cyclin D1 and carcinoembryonic antigen.

Harbeck teaches measuring mRNA levels for breast tumor markers for urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor-1 (PAI-1) in combination with cyclin D1 and carcinoembryonic antigen to facilitate the classification of subjects into high and low risk subjects [0067; 0195] using biological samples like any body fluid of the subject including blood, serum, plasma, milk, urine, saliva, pleural effusions, synovial fluid, spinal fluid, tissue infiltrations and tumor infiltrates, cells, tissues and tissue extracts [0021; 0196] and core needle biopsy and body fluid aspiration from breast, where reagents for binding a nucleic acid (e.g., a mRNA, a spliced mRNA, a cDNA, or the like) include complementary nucleic acids, for

example, *molecular beacon probes* [0207]. Harbeck discloses a comparison of the prognostic value using the above markers to known Her2/neu tumor marker in scoring breast cancer [0243]. Harbeck teaches "Additionally, it is possible to measure the levels of uPA and PAI-1 using in situ assays, i.e., directly upon tissue sections (fixed and/or frozen, e.g., paraffin sections) of patient tissue obtained from biopsies or resections, (e.g., laser micro-dissection of single cells) such that no nucleic acid purification is necessary. Noack et al., 1999, Int. J. Oncol. 15(4):617-23. Nucleic acid reagents such as those described in Section 5.7 may be used as probes and/or primers for such in situ procedures (see, e.g., Nuovo, G. J., 1992, PCR In Situ Hybridization: Protocols And Applications, Raven Press, NY)" [0179]. Harbeck teaches and appreciates performing detection methods using the molecular beacon probes by directly contacting cells in situ which does not require mRNA extraction.

One skilled in the art would have been motivated to have produced and been assured of success in having produced the claimed tumor marker mRNA detection method based on the combined disclosures of Sokol and Harbeck. In order to visualize any tumor marker mRNA by fluorescence signal detection in a cell sample from a cancer patient against a control condition, the ordinary artisan would have found more than sufficient motivation in Sokol's in situ whole cell methods to introduce one or more molecular beacon probes labeled with different colored fluorphors in order to differentiate different mRNA targets for tumor marker mRNAs. Sokol's methodology is based on detecting a tumor marker mRNA for rav oncogene in a human cancer cell line, thus to extend this method to detecting other tumor markers within the same cell line or

to other kinds of samples where other tumor marker mRNAs where expressed would have been obvious in view of Harbeck. Harbeck teaches and appreciates different methods for detecting breast cancer-associated tumor marker mRNAs by different means including MBs and that this could be performed by in situ hybridization on tissue samples. Thus the ordinary artisan could have detected not only rav oncogene but other tumor marker mRNAs for urokinase-type plasminogen activator (uPA), plasminogen activator inhibitor-1 (PAI-1), cyclin D1 and carcinoembryonic antigen and Her2/neu based on the combined teachings of Sokol and Harbeck using the MB strategy where both references taught and appreciated the sensitivity and targeting specificity of these fluorescence-based reagents. The ordinary artisan could have used cells from breast lavage, aspiration or needle biopsy and from breast cancer samples based on Harbeck's disclosure to measure mRNA by in situ hybridization in these samples. The ordinary artisan would have been assured of success in modifying the method of Sokol to detect other tumor marker mRNAs as taught by Harbeck because Sokol provides the guidance for selecting tumor-marker specific MBs and the control conditions for performing the intracellular assay to visualize and quantitate the fluorescent detection of tumor marker mRNAs. The claimed method was prima facie obvious at the time of the invention over Sokol and Harbeck.

human chorionic gonadotropin

26. Claims 1-5 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sokol et al. (PNAS 95:11538-11543 (1998); cited in the IDS of 2/21/08) as applied to

claim1 above, and further in view of Span et al. (Clin. Chem. 49(7); 1074-1080 (July 2003); cited in the PTO 892 form of 2/5/08).

The interpretation of Claims 1-5 is discussed above. Claim 2 is further drawn to detecting mRNA for human chorionic gonadotropin and Claim 3 is drawn to a tissue.

The claimed method was prima facie obvious at the time of the invention over Sokol in view of Span.

The interpretation of Sokol is discussed above under section 25. Sokol teaches and appreciates that MBs can be used to study many aspects of RNA biology in different kinds of cells, but does not specifically teach detecting markers for human chorionic gonadotropin.

Span discloses a method for detecting human choroinic gonadotrophin-β-3, 5 and 8 mRNAs in human breast tissues using molecular beacon probes, for example, the design of which is described on p. 1075, Col. 2, ¶4: "A probe (5'-FAM-cgcttccagtccaagcg-TAMRA-3', where FAM is 6-carboxyfluorescein and TAMRA is 6-carboxytetramethylrhodamine) that specifically annealed with transcripts from the hCG-β-3, 5 and 8 genes was designed essentially according to guidelines for Molecular Beacons described by Tyagi and Kramer (ref. 13)"..."However to obtain a smaller loop sequence, only one arm, complementary to five nucleotides at the 5' end of the hCG-β sequence was attached 3'." Biological samples were fresh frozen tissue from a tumor bank obtained from surgically resected breast tumors (p. 1075, Col. 1, ¶3) and a section would have been used on which to perform the assay (p. 1075, Col. 2, ¶2). The assay relies on a closed tube format using real-time fluorescence reverse transcription PCR

where the data are quantitative and linear over a wide range of template concentrations (p. 1079, Col. 2, ¶2). Concentration or amount of the hCG-β mRNAs in the tissues was determined following PCR amplification with the probe and found to have independent prognostic value for relapse free survival in sporadic breast cancer patients (Figure 1). Thus Span reads on the method steps of detecting, identifying and quantitating the presence of the tumor marker for human chorionic gonadotropin mRNA in breast tumor tissue sample.

One skilled in the art would have been motivated to have produced and been assured of success in having produced the claimed tumor marker mRNA detection method based on the combined disclosures of Sokol and Span. In order to visualize any tumor marker mRNA by fluorescence signal detection in a cell sample from a cancer patient against a control condition, the ordinary artisan would have found more than sufficient motivation in Sokol's in situ whole cell methods to introduce one or more molecular beacon probes labeled with different colored fluorphors in order to differentiate different mRNA targets for tumor marker mRNAs. Sokol's methodology is based on detecting a tumor marker mRNA for rav oncogene in a human cancer cell line, thus to extend this method to detecting other tumor markers within the same cell line or to other kinds of samples where other tumor marker mRNAs are expressed would have been obvious in view of Span. Span specifically teaches and appreciates detecting breast cancer-associated tumor marker mRNAs with an MB. Span relies on the additional step of RT-PCR to amplify the signal in breast tissue which Sokol does not.

The ordinary artisan would have found more than sufficient motivation to modify the MB of Span for introduction into the whole cell method of Sokol which did not rely on RT-PCR for amplification, yet which was shown to specifically and quantitatively yield fluorescent detection of tumor marker mRNA in situ. Thus the ordinary artisan could have detected not only rav oncogene but other tumor marker mRNAs like human chorionic gonadotropin mRNA based on the combined teachings of Sokol and Harbeck using the MB strategy where both references taught and appreciated the sensitivity and targeting specificity of these fluorescence-based reagents. The ordinary artisan could have used cells from breast tissue based on Span's disclosure to measure human chorionic gonadotropin mRNA with MBs. The ordinary artisan would have been assured of success in modifying the method of Sokol to detect other tumor marker mRNAs as taught by Span because Sokol provides the guidance for selecting tumor-marker specific MBs and the control conditions for performing the intracellular assay to visualize and quantitate the fluorescent detection of tumor marker mRNAs. The claimed method was prima facie obvious at the time of the invention over Sokol and Harbeck.

Her2/neu

27. Claims 1-3 and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sokol et al. (PNAS 95:11538-11543 (1998); cited in the IDS of 2/21/08) as applied to claim1 above, and further in view of Vijayanathan et al. (Antisense & Nuc. Acid drug Devel. 12:225-233 August 2002); cited in the PTO 892 form of 2/5/08).

The interpretation of Claims 1-3 and 14 is discussed above under section 25.

The claimed method was prima facie obvious at the time of the invention over Sokol in view of Vijayanathan.

The interpretation of Sokol is discussed above under section 25. Sokol teaches and appreciates that MBs can be used to study many aspects of RNA biology in different kinds of cells, but does not specifically teach detecting markers for Her2/neu.

Vijayanathan discloses using two molecular beacons (MB) to identify Her2/neu expression where Her2/neu overexpression is considered to be a negative prognostic marker for some tumors (p.226, Col. 1, ¶2), The first MB is composed of a 6-nt complementary flanking region at both ends and a 15-nt loop region comprising an antisense sequence capable of hybridizing to the AUG translational start site of the Her2/neu 2 mRNA. The second MB probe was a 32-mer composed of a 7-nt complementary flanking sequence at both ends and an 18-nt loop region. The donor and acceptor were fluorescein and 4-([4'-(dimethylamino) phenyl]azo)benzoic acid, respectively. Vijayanathan teaches that the method is qualitative because the MB can "readily distinguish targets differing by a single nucleotide" and quantitative because the MB can detect nanomolar concentrations of target substrate and further because the assay can be performed within a cell in real-time setup (p. 231, Col. 1, ¶1). Thus Vijayanathan reads on the method steps of detecting, identifying and quantitating the presence of the tumor marker for Her2/neu mRNA in cell samples from tumors.

One skilled in the art would have been motivated to have produced and been assured of success in having produced the claimed tumor marker mRNA detection method based on the combined disclosures of Sokol and Vijayanathan. In order to

visualize any tumor marker mRNA by fluorescence signal detection in a cell sample from a cancer patient against a control condition, the ordinary artisan would have found more than sufficient motivation in Sokol's in situ whole cell methods to introduce one or more molecular beacon probes labeled with different colored fluorphors in order to differentiate different mRNA targets for tumor marker mRNAs. Sokol's methodology is based on detecting a tumor marker mRNA for rav oncogene in a human cancer cell line, thus to extend this method to detecting other tumor markers within the same cell line or to other kinds of samples where other tumor marker mRNAs are expressed would have been obvious in view of Vijayanathan. Vijayanathan specifically teaches and appreciates detecting a tumor marker mRNA, Her2/neu, with a MB. Vijayanathan relies on the additional step of RT-PCR to amplify a synthetic target mRNA substrate for Her2/neu which Sokol does not.

The ordinary artisan would have found more than sufficient motivation to modify the MB of Vijayanathan for introduction into the whole cell method of Sokol which did not rely on RT-PCR for amplification, yet which was shown to specifically and quantitatively yield fluorescent detection of tumor marker mRNA in situ. Thus the ordinary artisan could have detected not only rav oncogene but other tumor marker mRNAs like Her2/neu mRNA based on the combined teachings of Sokol and Vijayanathan using the MB strategy where both references taught and appreciated the sensitivity and targeting specificity of these fluorescence-based reagents. The ordinary artisan could have used cells from any source based on Sokol's disclosure. The ordinary artisan would have been assured of success in modifying the method of Sokol

to detect other tumor marker mRNAs as taught by Vijayanathan because Sokol provides the guidance for selecting tumor-marker specific MBs and the control conditions for performing the intracellular assay to visualize and quantitate the fluorescent detection of tumor marker mRNAs. The claimed method was prima facie obvious at the time of the invention over Sokol and Vijayanathan.

K-ras mutant

28. Claims 1-4 and 16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sokol et al. (PNAS 95:11538-11543 (1998); cited in the IDS of 2/21/08) as applied to claim1 above, and further in view of Singer et al. (Am. J. Pathol. 160(4):1223-1228 (April 2002); cited in the PTO 892 form of 2/5/08) as evidenced by Vogelstein et al. (PNAS 96:9236-9241 (1999)); cited in the PTO 892 form of 2/5/08).

The interpretation of Claims 1-4 is discussed above under section 25. Claims 2 and 16 are drawn to an mRNA for k-ras mutant. Claim 3 is further drawn to the sample from blood or urine.

The claimed method was prima facie obvious at the time of the invention over Sokol in view of Singer as evidenced by Vogelstein.

The interpretation of Sokol is discussed above under section 25. Sokol teaches and appreciates that MBs can be used to study many aspects of RNA biology in different kinds of cells, but does not specifically teach detecting markers for k-ras mutant.

Singer teaches examining K-ras mutations at codon 12 and 13 using digital PCR and molecular beacons (MB) (p. 1225, Col. 2, ¶3) in paraffin-embedded tissue samples of ovarian serous tumors. Singer incorporates by reference the methodology of Vogelstein for digital PCR using MB for detecting K-ras mutants in colorectal cancer cell lines. Vogelstein teaches the nucleotide sequence for MB red and MB green for K-ras mutant at p. 9237, Col. 1, ¶2. Vogelstein teaches that the method is useful for detecting small numbers of mutant containing cancer cells in for example blood and urine (p. 9236, Col. 1, ¶1). Singer discloses detecting and identifying the K-ras mutants, and by incorporation to Vogelstein, quantitating tumor gene expression levels. Thus Singer as evidenced by Vogelstein reads on the method steps of detecting, identifying and quantitating the presence of the tumor marker for K-ras mutant gene in ovarian tumor tissue sample, blood or urine.

One skilled in the art would have been motivated to have produced and been assured of success in having produced the claimed tumor marker mRNA detection method based on the combined disclosures of Sokol and Singer as evidenced by Vogelstein. In order to visualize any tumor marker mRNA by fluorescence signal detection in a cell sample from a cancer patient against a control condition, the ordinary artisan would have found more than sufficient motivation in Sokol's in situ whole cell methods to introduce one or more molecular beacon probes labeled with different colored fluorphors in order to differentiate different mRNA targets for tumor marker mRNAs. Sokol's methodology is based on detecting a tumor marker mRNA for rav oncogene in a human cancer cell line, thus to extend this method to detecting other

tumor markers within the same cell line or to other kinds of samples where other tumor marker mRNAs are expressed would have been obvious in view of Singer as evidenced by Vogelstein. Singer specifically teaches and appreciates detecting a tumor marker mRNA, k-ras mutant, with a MB. Singer relies on the additional step of RT-PCR as evidenced by Vogelstein to amplify a target mRNA for k-ras mutants in cancer cell samples which Sokol does not.

The ordinary artisan would have found more than sufficient motivation to modify the MB of Singer or Vogelstein for introduction into the whole cell method of Sokol which did not rely on RT-PCR for amplification, yet which was shown to specifically and quantitatively yield fluorescent detection of tumor marker mRNA in situ. Thus the ordinary artisan could have detected not only rav oncogene but other tumor marker mRNAs like K-ras mutant mRNA based on the combined teachings of Sokol and Singer as evidenced by Vogelstein using the MB strategy where all three references taught and appreciated the sensitivity and targeting specificity of these fluorescence-based reagents. The ordinary artisan could have used cells from any source based on Sokol's disclosure. The ordinary artisan would have been assured of success in modifying the method of Sokol to detect other tumor marker mRNAs as taught by Singer as evidenced by Vogelstein because Sokol provides the guidance for selecting tumor-marker specific MBs and the control conditions for performing the intracellular assay to visualize and quantitate the fluorescent detection of tumor marker mRNAs. The claimed method was prima facie obvious at the time of the invention over Sokol and Singer as evidenced by Vogelstein.

survivin

29. Claims 1-3 and 10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sokol et al. (PNAS 95:11538-11543 (1998); cited in the IDS of 2/21/08) as applied to claim1 above, and further in view of Wen et al. (Cancer Gene Ther. 7(11):1469-1480 (2000); cited in the PTO 892 form of 2/5/08).

The interpretation of Claims 1-3 is discussed above under section 25. Claims 2 and 10 are drawn to an mRNA for survivin.

The claimed method was prima facie obvious at the time of the invention over Sokol in view of Wen.

The interpretation of Sokol is discussed above under section 25. Sokol teaches and appreciates that MBs can be used to study many aspects of RNA biology in different kinds of cells, but does not specifically teach detecting markers for survivin.

Wen discloses methods for measuring and quantitating survivin mRNA with molecular beacon probes labeled with a reporter fluorescent dye [FAM (6-carboxy-fluorescein)] at the 5-' end and a quencher fluorescent dye TAMRA (6-carboxy-tetramethyl-rhodamine) at the 3'- end when added to a real-time PCR reaction. Wen teaches that when the probe is intact, the fluorescence emission of the reporter is quenched due to the physical proximity of the reporter and quencher fluorescent dyes (p. 1471, Col. 2, ¶1). Wen teaches detecting survivin and GAPDH mRNA from biopsy samples which are obtained from xenografted human breast cancer cell lines grown in mice in vivo as established tumors (p. 1447, Col. 2, ¶2). Thus Wen reads on the method

steps of detecting, identifying and quantitating the presence of the tumor marker for surivin (and house keeping gene for GAPDH) in human breast tumor tissue sample.

One skilled in the art would have been motivated to have produced and been assured of success in having produced the claimed tumor marker mRNA detection method based on the combined disclosures of Sokol and Wen. In order to visualize any tumor marker mRNA by fluorescence signal detection in a cell sample from a cancer patient against a control condition, the ordinary artisan would have found more than sufficient motivation in Sokol's in situ whole cell methods to introduce one or more molecular beacon probes labeled with different colored fluorphors in order to differentiate different mRNA targets for tumor marker mRNAs. Sokol's methodology is based on detecting a tumor marker mRNA for rav oncogene in a human cancer cell line, thus to extend this method to detecting other tumor markers within the same cell line or to other kinds of samples where other tumor marker mRNAs are expressed would have been obvious in view of Wen. Wen specifically teaches and appreciates detecting a tumor marker mRNA, survivin, with a MB. Wen relies on the additional step of RT-PCR to amplify a target mRNA for survivin in cancer cell samples which Sokol does not.

The ordinary artisan would have found more than sufficient motivation to modify the MB of Wen for introduction into the whole cell method of Sokol which did not rely on RT-PCR for amplification, yet which was shown to specifically and quantitatively yield fluorescent detection of tumor marker mRNA in situ. Thus the ordinary artisan could have detected not only rav oncogene but other tumor marker mRNAs like surviving mRNA based on the combined teachings of Sokol and Wen using the MB strategy

where both taught and appreciated the sensitivity and targeting specificity of these fluorescence-based reagents. The ordinary artisan could have used cells from any source based on Sokol's disclosure. The ordinary artisan would have been assured of success in modifying the method of Sokol to detect other tumor marker mRNAs as taught by Wen because Sokol provides the guidance for selecting tumor-marker specific MBs and the control conditions for performing the intracellular assay to visualize and quantitate the fluorescent detection of tumor marker mRNAs. The claimed method was prima facie obvious at the time of the invention over Sokol and Wen.

Conclusion

30. No claims are allowed.

31. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of

the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

32. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lynn Bristol whose telephone number is 571-272-6883. The examiner can normally be reached on 8:00-4:00, Monday through Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Larry Helms can be reached on 571-272-0832. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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/David J Blanchard/
Primary Examiner, Art Unit 1643